Adenosine receptors and behavioral actions of methylxanthines

[caffeine/theophylline/N^6-cyclohexyladenosine/N^6-(phenylisopropyl)adenosine]

SOLOMON H. SNYDER*, JEFFERSON J. KATIMS*†, ZOLTAN ANNAU†, ROBERT F. BRUNS*‡, AND
JOHN W. DALY‡

*Departments of Neuroscience, Pharmacology and Experimental Therapeutics, Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine;
†Departments of Environmental Health Sciences, Johns Hopkins School of Hygiene, Baltimore, Maryland 21205; and ‡Laboratory of Bioorganic Chemistry,
National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20205

Contributed by Solomon H. Snyder, February 6, 1981

ABSTRACT   Central stimulant actions of 10 methylxanthines
in mice correlate with affinities for adenosine receptors labeled
with N^6-[^{3}H]cyclohexyladenosine. Affinities of methylxanthines
for adenosine receptors are consonant with central levels attained
at behaviorally effective doses. The much higher concentrations
of methylxanthines required to influence benzodiazepine receptor
binding do not correlate with behavioral potency. N^6-L-Phenyl-
isopropyl)adenosine (L-PIA), a metabolically stable analog of aden-
osine with high affinity for adenosine receptors, is an extremely
potent behavioral depressant, reducing locomotor activity of mice
at doses as little as 0.05 μmol/kg. The D isomer, which has much
less affinity for adenosine receptors, is much less active as a central
depressant. Theophylline stimulates locomotor activity and re-
verses depressant effects of L-PIA. Caffeine or 1,7-dimethylxan-
thine, when administered alone, elicits biphasic effects, with lo-
comotor depression at lower doses and stimulation at higher doses.
When administered with L-PIA, even low doses of caffeine pro-
duce marked stimulation. 3-Isobutyl-1-methylxanthine given alone
elicits only behavioral depression. However, like theophylline and
caffeine, isobutylmethylxanthine reverses the L-PIA-evoked
depression, converting it into pronounced locomotor stimulation.
The data strongly suggest that the behavioral stimulant effects of
methylxanthines involve a blockade of central adenosine receptors.

Although methylxanthines such as caffeine and theophylline are
among the most widely used behavioral stimulant substances,
molecular mechanisms for their stimulant effects are unclear.
Methylxanthines can inhibit phosphodiesterase, and thus pre-
vent inactivation of cyclic AMP (1), but the concentrations of
caffeine and theophylline required to inhibit phosphodiester-
ases are substantially greater than those which occur in brain
at behaviorally effective doses (2, 3). Moreover, several potent
phosphodiesterase inhibitors lack behavioral stimulant actions
and indeed are central depressants (4). Adenosine receptor ac-
tivity is blocked by methylxanthines in concentrations similar
to those that occur after stimulant doses (5, 6). Because the gen-
eral neurophysiologic actions of adenosine are inhibitory (7), it
is conceivable that methylxanthines exert stimulant actions by
blocking adenosine effects.

In several attempts to measure binding of adenosine-related
ligands to membranes, binding sites largely lacked the speci-
ficity of physiologic adenosine receptors (8–12). Recently, we
(13, 14) and others (15, 16) have demonstrated binding of ^{3}H-
labeled ligands to adenosine receptors in brain and testes (13,
17).

In the present study we show a correlation between potencies
of a series of methylxanthines in stimulating locomotor activity
of mice and in competing at adenosine receptors labeled with
N^6-[^{3}H]cyclohexyladenosine ([^{3}H]CHA). Both CHA and N^6-(L-
phenylisopropyl)adenosine (L-PIA), stable and potent adeno-

sine analogs, are shown to be extremely potent behavioral de-
pressants. Low doses of xanthines dramatically reverse the L-
PIA-evoked depression.

MATERIALS AND METHODS

Biochemical. [^{3}H]CHA binding to whole mouse brain mem-
branes was assayed as reported (13). Properties of [^{3}H]CHA
binding to mouse brain membrane were essentially the same
as for guinea pig brain (13). [^{3}H]Flunitrazepam binding to
mouse brain membranes was assayed as before (18).

[^{3}H]CHA (14 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and
[^{3}H]flunitrazepam (79 Ci/mmol) were obtained from New En-
gland Nuclear. The sources of xanthines were as described (13).

Behavioral. Naive adult male ICR mice (25–40 g) from Blue
Spruce Farms (Altamont, NY) were given food and water ad lib.
The mice were housed 20 per cage in 26 × 46 × 17 cm poly-
propylene cages and were exposed to a 12-hr/12-hr light/dark
cycle (lights on, 0700). Mice were permitted to adapt to their
housing for a minimum of 48 hr before testing. Behavioral tests
were performed every 4 weeks, new control groups were es-
tablished. Unless stated otherwise, all drugs were administered
as a saline solution given intraperitoneally at 10 μl/g of body
weight:

Mice received drugs 10 min prior to the 1-hr locomotor ac-
tivity testing period and were placed individually in holding
cages containing a sawdust bedding similar to that of their group
cages.

Locomotor activity data were subjected to parametric statis-
tical analysis by using repeated measures three-way analysis of
variance and covariance with the least-squares computation for
unequal numbers. Two independent subjects grouping factors
consisted of the cage and the various doses in which they were
administered. The repeated measures of spontaneous locomo-
tor activity during a testing session were considered the within-
subject dependent repeated measure. This analysis of variance
was repeated with a logarithmic transformation of the data. Stu-
dent's t test was used for making individual comparisons.

Locomotor activity was measured in four identical automated
38 × 38 × 38 cm open-field devices built, in our laboratories,
of black Plexiglas. The ceiling of white Plexiglas concealed a 6-
W fluorescent light fixture to provide background illumination
and an exhaust fan for ventilation. Sixty-four cadmium sulfide
photosensitive devices were placed under the transparent Plex-
iglas floor 3.8 cm apart in an 8×8 array and connected to an Intel
microcomputer that monitored the state and location of the
photosensitive elements 10 times per sec. The printout (Tele-
type 43) showed the accumulated time on each of the 64 pho-
tocells for the predetermined time period, the number of pho-

Correction: Proc. Natl. Acad. Sci. USA Vol. 78(5) May 1981

tocells covered during the interval, and cell changes from the active to the inactive state.

RESULTS

Effects of Methylxanthines on Locomotor Stimulation and on Adenosine and Benzodiazepine Receptor Binding. Methylxanthines increase locomotor activity of rodents (19) but much less consistently than do amphetamines. Because preliminary open-field studies failed to show consistent locomotor stimulation with caffeine, we utilized a photoelectric activity meter with 64 sensors. Drugs such as caffeine and theophylline enhanced locomotor activity up to 4-fold compared to saline controls (Fig. 1; Table 1). Caffeine and 1,7-dimethylxanthine reduced locomotor activity at lower doses (5 and 10 μmol/kg) but stimulated activity at 30 and 100 μmol/kg. By contrast, no locomotor depression occurred with any dose of theophylline, 7-(β-chloroethyl)theophylline, or 7-(β-hydroxyethyl)theophylline. Isocaffeine and 3-isobutyl-1-methylxanthine (IBMX) moderately depressed activity at all doses. Theobromine, 8-chlorotheophylline, and 1,9-dimethylxanthine had negligible influence on locomotor activity at all doses.

Brain levels of the alkylxanthines were assessed 30 min after a 100-μmol/kg dose (Table 1). Caffeine and theophylline levels were about 60 μM. Theobromine, 1,7-dimethylxanthine, 7-(β-hydroxyethyl)theophylline, and 7-(β-chloroethyl)theophylline had levels of about 20–30 μM; IBMX, 8-chlorotheophylline and isocaffeine levels were 10–15 μM.

In general, stimulant potencies of the methylxanthines correlated with potencies in competing for the adenosine receptor labeled with [^{3}H]CHA. The three methylxanthines that were most potent at [^{3}H]CHA sites also were the most potent locomotor stimulants. The four xanthines that were weakest in competing for [^{3}H]CHA binding also were weakest in eliciting locomotor stimulation. Three of these—8-chlorotheophylline, 1,9-dimethylxanthine, and isocaffeine—did not penetrate well into brain. However, bioavailability cannot account for the differences in behavioral effects. Thus, 7-(β-chloroethyl)theophylline, which was most potent behaviorally, had one of the lowest brain levels. Theobromine, which was be-

haviorally inactive, had brain levels as high or higher than 1,7-dimethylxanthine and 7-(β-chloroethyl)theophylline, which were behaviorally active.

It has been suggested that stimulant effects of methylxanthines might be attributable to blockade of benzodiazepine receptors (21). However, the behaviorally potent methylxanthines are about 100 times more potent at adenosine than benzodiazepine receptors and no correlation exists between behavioral potencies and effects at benzodiazepine receptors.

Influences of L-PIA on Mouse Locomotor Activity and Interactions with Methylxanthines. Although potencies of xanthines as stimulants largely correlate with their potencies in competing for adenosine receptors, there is one notable exception. IBMX was as potent as caffeine at adenosine receptors yet failed to stimulate activity and, in fact, elicited locomotor depression. Unlike the other xanthines, IBMX is a potent phosphodiesterase inhibitor (22), and phosphodiesterase inhibitors are usually central depressants (4). Another difficulty in analyzing behavioral effects of xanthines is the biphasic action of agents such as caffeine and 1,7-dimethylxanthine, which reduced and stimulated behavioral activity at low and high doses, respectively. Theophylline failed to display behavioral depression at any dose examined. Differential stimulant and depressant potencies of various methylxanthines might obscure their intrinsic stimulant potencies.

To evaluate behavioral actions of the methylxanthines on systems specifically regulated by adenosine, we explored effects of PIA. In an earlier study, PIA elicited behavioral depression in rats (23). We examined in detail influences of both L and D-PIA (Fig. 2). CHA and L-PIA both were very potent in eliciting locomotor depression. The fact that L-PIA is more potent than D-PIA suggests that these effects involve adenosine A_1-receptors, which display marked stereospecificity for isomers of PIA, rather than A_2-receptors, at which L- and D-PIA have nearly equal potencies (13, 24). At doses as little as 0.1 μmol/kg, CHA or L-PIA markedly reduced locomotor activity of mice, and significant depression was detected at 0.05 μmol/kg. These doses, around 20 μg/kg, indicate that N^6-substituted adenosines rank
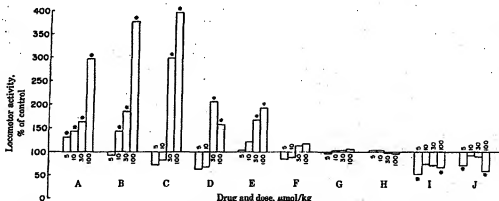

FIG. 1. Effect of alkylxanthines on locomotor activity of mice. Locomotor activity values for groups of 10–20 mice at each dose are for the second 30 min after intraperitoneal injections of the indicated doses except for 7-(β-chloroethyl)theophylline for which the activity values represent the first 30-min period. Values represent the locomotor activity as percentage of the activity of saline-injected control mice and are presented as the antilogarithm of a logarithmic transformation of this data. An overall analysis of variance revealed a significant drug group effect ($F = 9.93$; $df = 6251$; $P < 0.001$), a significant drug × dose interaction ($F = 2.78$, $df = 18,251$; $P < 0.001$), and a significant drug × time interaction ($F = 5.71$, $df = 90,125$; $P < 0.001$). Similar levels of significance were obtained for a logarithmic transformation of this data. *, Significantly different from saline, $P < 0.005$ by Student's t test. A, 7-(β-chloroethyl)theophylline; B, theophylline; C, caffeine; D, 1,7-dimethylxanthine; E, 7-(β-hydroxyethyl)theophylline; F, theobromine; G, 8-chlorotheophylline; H,1,9-dimethylxanthine; I, isocaffeine; J, 3-isobutyl-1-methylxanthine.

Table 1. Xanthines: Behavioral stimulant potencies and effects on adenosine and benzodiazepine receptor binding and brain levels

	Receptor binding IC$_{50}$, µM		Locomotor stimulation threshold, µmol/kg	Brain concentration, µM
Xanthine	[^{3}H]Flunitrazepam	[^{3}H]CHA		
1. 7(β-Chloroethyl)theophylline	900	10	5	19 ± 2 (3)
2. Theophylline	2000	23	10	58 ± 12 (5)
3. 1,7-Dimethylxanthine	2000	30	30	26 ± 8 (5)
4. 3-Isobutyl-1-methylxanthine (IBMX)	≈100	50	>50	15 ± 3 (3)
5. Caffeine	800	50	30	63 ± 13 (5)
6. 7(β-Hydroxyethyl)theophylline	2000	100	100	32 ± 2 (2)
7. Theobromine	>2000	150	>100	29 ± 3 (3)
8. 8-Chlorotheophylline	>2000	500	>250	13 ± 6 (3)
9. 1,9-Dimethylxanthine	>2000	>1000	>250	—
10. Isocaffeine	1000	>1000	>250	11 ± 5 (3)

Binding of [^{3}H]CHA (1.0 nM) or [^{3}H]flunitrazepam (0.2 nM) was assayed in triplicate with six concentrations of xanthines. Data are means of three determinations of IC$_{50}$ values (concentration to inhibit specific binding by 50%) which varied less than 20%. Locomotor stimulation threshold represents the minimal dose to augment monitored locomotor activity significantly (tested by statistical analyses). For each methylxanthine, five or six doses from 2.5 to 250 µmol/kg were evaluated with 10–20 mice at each dose. At 250 µmol of IBMX per kg, most mice died. To measure methylxanthine brain levels, mice were given 100-µmol/kg doses and were killed 30 min later. Brains were homogenized with 5 vol of 0.01 M HCl and extracted three times with 10 vol of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dissolved in 1 vol of solvent for high-pressure liquid chromatography adapted from the method of Blanchard *et al.* (20). A LiChrosorb 18 (4.6 × 250 mm) reversed-phase column (Altex Scientific, Berkeley, CA) was used with 0.01 M acetate buffer, pH 4/acetonitrile, 90:10 (vol/vol), as solvent for xanthines 3, 7, and 10, an 85:15 mixture for xanthines 2, 5, 6, 8, and 9, and a 70:30 mixture for xanthines 1 and 4. A flow rate of 1 ml/min gave the following retention times (min) for the 10 xanthines: 1, 2.4; 2, 5.5; 3, 11.2; 4, 7.6; 5, 9.0; 6, 13.0; 7, 7.1; 8, 10.2; 9, 4.5; 10, 7.6. The injection volume was 20 µl. The ultraviolet detector was set at 273 nm, and integrated peak heights were compared to those of standard solutions of methylxanthines. All values are means ± SEM; the number of mouse brains is shown in parentheses. All data are corrected for recoveries of standards from control brains. Recovery of 1,9-dimethylxanthine was less than 5% and levels of this xanthine could not be determined but appeared to be less than 10 µM. Recoveries for the other xanthines were: 1, 90%; 2, 55%; 3, 56%; 4, 100%; 5, 90%; 6, 47%; 7, 70%; 8, 33%; 10, 32%.

among the most potent psychoactive drugs, comparable to LSD and the very potent butyrophenone neuroleptic spiperone (25).

At 0.2 µmol of L-PIA per kg, the mice displayed virtually no spontaneous motor activity at 30 min and were flaccid with their fore and hind limbs splayed. However, at this dose the animals were alert, responded to nociceptive stimuli such as tail pinching, and had intact righting and corneal reflexes. At this dose both tail and ears displayed a reddish coloration indicative of vasodilation. The respiratory rate was slowed, and respirations seemed to be greater. At 5 µmol/kg or higher, the righting reflex was abolished, although the animals were still awake. At progressively increasing doses up to 600 µmol/kg the mice still were alert but flaccid. The absence of lethality at doses thousands of times greater than behaviorally active doses suggests that behavioral effects are not related to systemic effects such as hypotension. After peripheral administration of [^{3}H]CHA to mice, its tissue concentrations are such that a 0.2-µmol/kg dose would give brain levels of 20 nM, several times greater than the K_d, 6nM for adenosine receptors (unpublished data). To further

ensure that L-PIA depression is centrally mediated, we showed that 8-(p-sulfophenyl)theophylline (60 mg/kg), which is as potent at theophylline at adenosine receptors (Table 1, but polar and not likely to enter the brain, failed to reverse L-PIA behavioral depression.

To explore possible interactions between L-PIA and methylxanthines, we administered these substances alone or in combination at various doses (Fig. 3). At each dose L-PIA alone depressed motor activity; at 30 and 100 µmol/kg it was a stimulant. Combining a "depressant" dose of caffeine (10 µmol/kg) with L-PIA markedly enhanced locomotor activity. Theophylline did not depress activity at any dose examined. The combination of theophylline (10 µmol/kg) and L-PIA produced considerably greater enhancement of locomotor activity than occurred with the same dose of theophylline alone. At 5–100 µmol/kg, IBMX alone failed to enhance locomotor activity and, in fact, depressed activity at most time points. L-PIA (0.2 µmol/kg) also depressed activity. Strikingly, the combination of IBMX (5 µmol/kg) and L-PIA, like combinations of L-PIA with either caffeine or theophylline, greatly augmented locomotor activity, to 300% of control activity at 60 min. A similar although less-pronounced reversal of L-PIA depression occurred at 2.5 µmol of IBMX per kg. These findings suggest that IBMX indeed possesses intrinsic stimulant activity that normally is masked by its separate depressant effects and unmasked by the interaction with L-PIA.

These dramatic interactions of methylxanthines with L-PIA are quite selective. No synergistic stimulation of locomotor activity occurred when amphetamine was combined with L-PIA (Fig. 3B). At 2.5 µmol/kg, amphetamine markedly augmented locomotor activity. A combination of this dose with L-PIA (0.2 µmol/kg) resulted in absence of stimulation or depression of activity.

Influences of L-PIA on Nociception and Drug-Induced Convulsions. The very potent and stereospecific behavioral effects of PIA and CHA suggest that these substances act upon adenosine A$_1$-receptors in the brain and may reflect the role of endogenous adenosine in the brain. Accordingly, we evaluated a
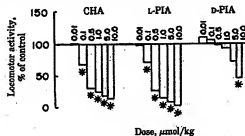

FIG. 2. Effects of CHA and L- or D-PIA on locomotor activity of mice. Locomotor activity for groups of nine mice at each intraperitoneal dose for the 20- to 30-min period after drug administration are expressed as percentage of activity of saline-injected controls. Values presented are for a logarithmic transformation of this data as in Fig. 1. *Significantly different from saline, $P < 0.005$ by Student's *t* test.
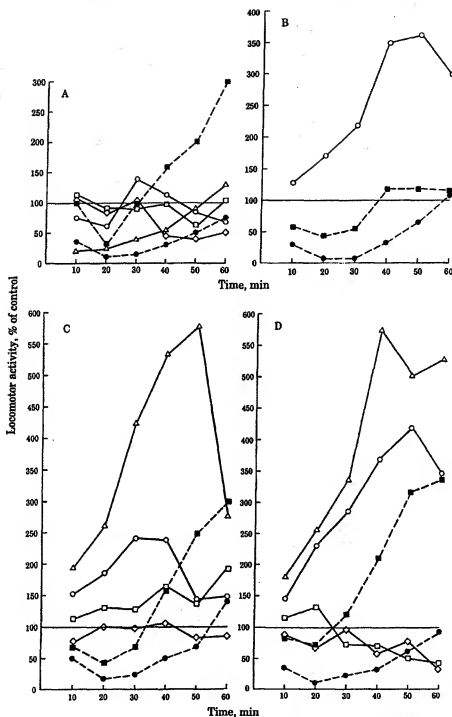
FIG. 3. Interactive effects of alkylxanthines or *d*-amphetamine and L-PIA on mouse locomotor activity. Mean values for groups of 10–15 mice at each intraperitoneal dose are expressed as percentage of saline injected controls. L-PIA and methylxanthines were given intraperitoneally at the same time; 20 min later, the mice were placed in activity-monitoring cages. (*A*) IBMX. ○, 5 μmol/kg; □, 10 μmol/kg; △, 30 μmol/kg; ●, 100 μmol/kg; ■, L-PIA at 0.2 μmol/kg; ▲, L-PIA at 0.2 μmol/kg plus IBMX at 5 μmol/kg. (*B*) d-Amphetamine. ○, 2.5 μmol/kg; ●, L-PIA at 0.2 μmol/kg; ■, L-PIA at 0.2 μmol/kg plus amphetamine at 2.5 μmol/kg. (*C*) Theophylline. Open symbols, doses as in *A*; ●, L-PIA, 0.15 μmol/kg; ■, L-PIA at 0.15 μmol/kg plus theophylline at 10 μmol/kg. (*D*) Caffeine. Open symbols, doses as in *A*; ●, L-PIA, 0.15 μmol/kg; ■, L-PIA at 0.2 μmol/kg plus caffeine at 10 μmol/kg.

possible role of adenosine in nociceptive and convulsant systems.

Convulsions were elicited in groups of 15 mice by administration of strychnine (5 μmol/kg) or pentylenetetrazole (0.4 mmol/kg). Diazepam at 5 mg/kg completely prevented both strychnine and pentylenetetrazole convulsions. L-PIA (10 μmol/kg) reduced the two types of convulsions by 50%. However, at 5 μmol/kg or lower, L-PIA lacked anticonvulsant effects despite its profound behavioral actions. In the tail flick test (26) in groups of 15 mice, L-PIA (5–10 μmol/kg) displayed some antinociceptive effects that were blocked by caffeine (0.2 mmol/kg) but not by naloxone (3 μmol/kg). Thus, whereas L-PIA displays some anticonvulsant and antinociceptive activity, these actions require doses at least 10 times the effective dose for behavioral depression.

DISCUSSION

The present study strongly suggests that the behavioral stimulant effects of methylxanthines involve blockade of adenosine receptors. Potencies of methylxanthines in competing at adenosine receptor binding sites correlate with locomotor stimulation. The failure of the potent adenosine receptor blocker IBMX to stimulate locomotor activity directly may reflect a "contaminating" behavioral depressant effect, perhaps related to phosphodiesterase inhibition. The conversion, by low IBMX doses, of L-PIA-induced depression into a pronounced behavioral activation suggests an intrinsic stimulant activity of IBMX. Although certain of the behaviorally inactive methylxanthines display reduced brain penetration, variations of bioavailability do not account for differences in behavioral potency, and brain levels of most methylxanthines are sufficient to occupy adenosine receptors.

There appear to exist at least two distinct adenosine receptors. A_1-sites are associated with decreases in cyclic AMP levels, are influenced by nanomolar concentrations of adenosine and related agents, and respond stereoselectively to PIA (13, 27–29). A_2-receptors, on the other hand, are associated with augmentation of cyclic AMP levels, respond to micromolar concentrations of adenosine, and do not markedly differentiate between the isomers of PIA. In the present study, we evaluated effects of xanthines only on A_1-receptors labeled by [³H]CHA. Previously, we labeled apparent A_2-receptors in brain membranes with 1,3-[³H]diethyl-8-phenylxanthine (13). Most of the methylxanthines used in the present study have similar potencies on adenosine receptors labeled with [³H]diethylphenylxanthine and [³H]CHA. Because PIA-induced depression, which is strikingly reversed by methylxanthines, appears to be stereoselective, it seems probable that stimulant effects of methylxanthines involve blockade of A_1-receptors. However, a role for A_2-receptors cannot be excluded. The extremely potent locomotor depressant but nonhypnotic actions of PIA suggest that adenosine analogs may exert useful, possibly therapeutic, behavioral effects.

1. Butcher, R. W. & Sutherland, E. W. (1962) *J. Biol. Chem.* **237**, 1244–1250.
2. Fredholm, B. B., Fuxe, K. & Agnati, L. (1976) *Eur. J. Pharmacol.* **38**, 31–38.
3. Smellie, F. W., Davis, C. V., Daly, J. W. & Wells, J. N. (1979) *Life Sci.* **24**, 2475–2482.
4. Beer, B., Chasin, M., Clody, D. E., Voge, J. R. & Horovitz, Z. P. (1972) *Science* **176**, 428–430.
5. Nichols, R. E. & Walaszek, E. J. (1963) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **22**, 308.
6. Sattin, A. & Rall, T. W. (1970) *Mol. Pharmacol.* **6**, 13–23.
7. Phillis, J. W., Edstrom, J. P., Kostopoulos, G. K. & Kirkpatrick, J. R. (1979) *Can. J. Physiol. Pharmacol.* **57**, 1289–1312.
8. Malbon, C. C., Hert, R. C. & Fain, J. N. (1978) *J. Biol. Chem.* **253**, 3114–3122.
9. Dutta, P. & Mustafa, S. J. (1979) *J. Pharmacol. Exp. Ther.* **211**, 496–501.
10. Schwabe, U., Kiffe, H., Puchstein, C. & Trost, T. (1979) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **310**, 59–67.
11. Newman, M. E., DeLucia, R., Patel, J. & McIlwain, H. (1980) *Biochem. Soc. Trans.* **8**, 141–142.
12. Wu, P. H., Phillis, J. W., Balls, K. & Rinaldi, B. (1980) *Can. J. Physiol. Pharmacol.* **58**, 576–579.
13. Bruns, R. F., Daly, J. W. & Snyder, S. H. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5547–5551.
14. Snyder, S. H., Bruns, R. F., Daly, J. W. & Innis, R. B. (1981) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **40**, 142–146.
15. Williams, M. & Risley, E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6892–6896.
16. Schwabe, U. & Trost, T. (1980) *Naunyn-Schmiedeberg's Arch. Pharmacol.* **313**, 179–187.
17. Murphy, K. M. & Snyder, S. H. (1981) *Life Sci.* **28**, 917–920.
18. Chang, R. S. L. & Snyder, S. H. (1978) *Eur. J. Pharmacol.* **48**, 295–299.
19. Thithapanda, A., Maling, H. M. & Gillette, J. R. (1972) *Proc. Soc. Exp. Med.* **139**, 582–586.
20. Blanchard, J., Mohammadi, J. B. & Conrad, K. A. (1980) *Clin. Chem.* **26**, 1351–1354.
21. Skolnick, P., Paul, S. M. & Marangos, P. J. (1980) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 3050–3055.
22. Beavo, J. A., Rogers, N.-L., Crofford, O. B., Hardman, J. G., Sutherland, E. W. & Newman, E. V. (1970) *Mol. Pharmacol.* **6**, 597–603.
23. Vapaatalo, H., Onken, D., Neuronen, P. & Westerman, E. (1975) *Arzneim-Forsch.* **25**, 407–410.
24. Smellie, F. W., Daly, J. W., Dunwiddie, T. V. & Hoffer, B. J. (1979) *Life Sci.* **25**, 1739–1748.
25. Janssen, P. A. J. & Van Bever, W. F. M. in *Handbook of Psychopharmacology*, eds. Iversen, L. L., Iversen, S. D. & Snyder, S. H. (Plenum, New York), Vol. 10, pp. 1–36.
26. D'Amour, F. E. & Smith, D. L. (1941) *J. Pharmacol. Exp. Ther.* **72**, 74–79.
27. Van Calker, D., Muller, M. & Hamprecht, B. (1979) *J. Neurochem.* **33**, 999–1005.
28. Londos, C., Cooper, D. M. F. & Wolff, J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2551–2554.
29. Daly, J. W., Bruns, R. F. & Snyder, S. H. (1981) *Life Sci.* **28**, 2083–2097.